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(54) **Caries-preventive composition.**

(57) A caries-preventive composition comprises an antibody obtained by immunizing a mammal with at least one antigen selected from the group consisting of Streptococcus mutans, its cell-wall fraction, fibrous substance fraction, glucosyltransferase fraction and protein antigen fraction, and a synergist selected from the group consisting of fluorine compounds, chlorhexidine and its salts, lytic enzymes, bacteriocins, glucosyltransferase inhibitors, proteases and dextranases.

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"CARIES-PREVENTIVE COMPOSITION"

This invention relates to a caries-preventive composition which, when applied to the mouth, can prevent dental caries by suppressing formation of dental plaque.

Dental plaque firmly adhering to the surface of teeth is composed of about 70% bacteria, about 20% polysaccharides produced by the bacteria and about 10% food remains. It is said that acids stored in dental plaque decalcify enamel, causing dental caries. Therefore, dental plaque is observed as a cause of dental caries.

Formation of dental plaque is accelerated due to the synthesis of polysaccharides from sucrose by oral bacteria, especially Streptococcus mutans. In more detail, Streptococcus mutans synthesizes adhesive polysaccharides such as dextran and mutan from sucrose through the production of GTF (glucosyltransferase, dextran-synthesizing enzyme). The thus synthesized polysaccharides incorporate Streptococcus mutans as well as other bacteria (viruses), forming dental plaque having a given bacterial bouquet. In addition, bacteria such as Streptococcus mutans produce acids by utilizing various kinds of sugar and the thus produced acids decalcify the surface of enamel by remaining in polysaccharides and bacterial walls.

Accordingly, it is desirable to decrease the number of Streptococcus mutans in the mouth and suppress the formation of dental plaque in order to prevent dental caries.

It is known in British patent No.1,505,513 that colonization of Streptococcus mutans in the mouth is suppressed by using mother's milk obtained by immunizing a cow with whole bacterial bodies of Streptococcus mutans.

The present inventors studied antibodies which are amongst the antibodies to various antigens derived from Streptococcus mutans and inhibit the colonization of Streptococcus mutans in the mouth. As a result, the inventors found that antibodies contained in antiserum and milk obtained by immunizing mammals with Streptococcus mutans, its cell-wall fraction, fibrous substance fraction, glucosyltransferase fraction and protein antigen fraction have certain degrees of dental-plaque-formation suppressing effect. However, the effect was not necessarily sufficient and a higher effect of suppressing the formation of dental plaque was necessary.

An object of the present invention is to provide a caries-preventive composition having an excellent effect in preventing dental caries.

For the purpose of attaining the above object, the present inventors further conducted an intensive study, and, as a result, found that the combination

of such an antibody and a fluorine compound, chlorhexidine or a chlorhexidine salt, a lytic enzyme, a bacteriocin, a glucosyltransferase inhibitor, a protease or a dextranase works effectively for the prevention of dental caries by causing a significantly increased dental-plaque-formation suppressing effect through the suppression of colonization of Streptococcus mutans.

Therefore, this invention provides a caries-preventive composition characterized by being composed of the combination of antibody obtained by immunizing a mammal with at least one antigen selected from the group consisting of Streptococcus mutans, its cell-wall fraction, fibrous substance fraction, glucosyltransferase fraction and protein antigen fraction with at least one synergist selected from the group consisting of fluorine compounds, chlorhexidine and its salts, lytic enzymes, bacteriocins, glucosyltransferase inhibitors, proteases and dextranases.

According to this invention, since the combination of said antibody and said synergist component exerts a synergistic effect on the inhibition of colonization of Streptococcus mutans in the mouth, the formation of dental plaque is efficiently suppressed, resulting in the effective prevention of dental caries.

In addition, since said antibody and said synergist component both are quite safe, the caries-preventive composition of this invention can be safely used.

The above and other objects, features, and advantages of this invention will be more fully understood by reading the following description.

The caries-preventive composition according to this invention is prepared by use of antibody contained in antiserum and/or milk obtained by immunizing a mammal with at least one antigen selected from the group consisting of Streptococcus mutans, its cell-wall fraction, fibrous substance fraction, glucosyltransferase (GTF) fraction and protein antigen fraction as described above. It should be noted that the fibrous substance means a pili-like or fimbriae fraction.

Streptococcus mutans used as an antigen may be prepared through well-known culture and pretreatment carried out by, for example, growing bacteria in external solution obtained by the dialysis of BHI medium before the thus grown bacteria are washed and subjected to formalin treatment. Streptococcus mutans separated from human mouth and belonging to the serotypes C, D, E, F and G may preferably be used, particularly one belonging to the serotype-C which is numerous in the human mouth. Such Streptococcus mutans includes NCTC10449, Ingbritt, OMZ70, JC-2, etc. and their mutant strains.

The cell-wall fraction of Streptococcus mutans may be prepared, for example, according to the method of Bleiweis et al. (J. Bacteriol., 88, 1198-1200, 1964) by subjecting Streptococcus mutans to crushing treatment in a Brown's cell crusher and glass beads of 0.17 to

0.18 mm diameter, then treating the thus obtained cell walls with trypsin to remove protein contaminating the cell walls, followed by washing the cell walls with distilled water before they are lyophilized. The fibrous (pili-like or fimbriae) substance fraction may be prepared, for example, according to the method of J. Van Hoate et al. (Arch. Oral. Bio., 16, 1131-1141, 1971) by culturing Streptococcus mutans in a medium obtained by the dialysis of BHI medium and containing 5% sucrose under an anaerobic condition, then centrifuging the culture medium to obtain a supernatant solution, then adding three times as much ethanol as the supernatant solution by volume, followed by collecting the precipitate of the thus obtained solution. As the fibrous substance fraction there may also be used a pili-like structure from the cell wall of Streptococcus mutans and its purified substance prepared by the ordinary cell wall extract method from the cultured bacteria, using solvents such as phosphate buffer containing IM sodium chloride according to the method of Tsurumizu et al. (Japanese Journal of Bacteriology, 38, (1) 471, 1983). The GTF fraction may be prepared, for example, according to the method of Inoue et al. (Microbial Aspects of dental caries Vol. III, 665-682, 1976 [Information Retrieval Inc.]) using a solution prepared by the following method: after Streptococcus mutans is implanted and grown in a medium obtained by the dialysis of BHI medium, the bacterial bodies are removed by centrifugation and the supernatant is saturated with ammonium sulfate at the level

of 40%, followed by dialyzing the precipitate of the 40% ammonium sulfate fraction against 50 mM phosphate buffer solution and concentrating or diluting the obtained solution. The protein antigen fraction may be prepared, for example, according to the method of Lehner et al. (J. General Microbiology, 122, 217-225, 1981) by culturing Streptococcus mutans in a medium obtained by the dialysis of BHI medium, then centrifuging the culture medium to obtain a supernatant solution, followed by fractionation with 75% ammonium sulfate solution to collect the precipitate; the thus obtained precipitate is then subjected to DE-52 column chromatography under the existence of 6M urea, and the protein antigen fraction is dissolved in physiological saline, this being followed by dialyzing the thus obtained solution whereafter the dialyzed solution is subjected to gel filtration through Sepharose CL6B.

The usual method may be adopted in immunizing mammals with said antigens. As mammals to be immunized, goats, sheep, horses, cows or rabbits may be used.

The antibody (protein fraction in the antiserum and the milk) may be separated from the antiserum and the milk according to the ordinary antibody purification method including the salting-out method, the gel-filtration method, ion-exchange chromatography or affinity chromatography, the salting-out method using ammonium sulfate being preferred. In the salting-out method, the antiserum or the milk is saturated with ammonium sulfate, preferably at the level of not more than 40%, to produce

the precipitate, followed by dialyzing the precipitate against physiological saline to obtain the purified precipitate as the antibody. The preferred antibody is obtained from the equine antiserum and the bovine antiserum and milk.

In this invention, the antibody contained in the antiserum and milk obtained by immunizing the mammal with said antigen is blended into the composition. In this case, the antiserum and milk as well as the antibody separated and purified therefrom may be used. Each of these materials may be used alone or in a combination of two or more.

The caries-preventive composition according to this invention is prepared by the combination of said antibody and at least one synergist selected from the group consisting of fluorine compounds, chlorhexidine and its salts, lytic enzymes, bacteriocins, glucosyltransferase inhibitors, proteases and dextranases.

As fluorine compounds, sodium fluoride, potassium fluoride, lithium fluoride, ammonium fluoride, sodium monofluorophosphate, sodium hydrogen monofluorophosphate, potassium monofluorophosphate, ammonium monofluorophosphate, potassium hexafluorozirconate, and potassium hexafluorotitanate may be used. Also useful are cesium fluoride, nickel fluoride, zirconium fluoride, silver fluoride, hexylamine hydrofluoride, laurylamine hydrofluoride, cetylamine hydrofluoride, glycine hydrofluoride, lysine hydrofluoride, alanine hydrofluoride and the like. Among

them, monofluorophosphates such as sodium monofluorophosphate and potassium monofluorophosphate, alkali-metal fluorides such as sodium fluoride, potassium fluoride and ammonium fluoride, fluorides containing stannous tin such as stannous fluoride and stannous chloride fluoride and the like may preferably be used. Especially, sodium monofluorophosphate, sodium fluoride and stannous fluoride are more preferably used.

As chlorhexidine salts, chlorhexidine hydrochloride or chlorhexidine gluconate can be used.

As lytic enzymes, those derived from Streptomyces griseus, Streptomyces diastatochromagenes, Streptomyces farinosus, Chalaropsis, Flavobacterium, Myxobacter, Staphylococcus epidermidis,Micrococcus, Pseudomonas aetuginosa, Aeromanas, Streptomyces albus and Streptomyces globisporus can be used.

As bacteriocins, those derived from Enterobactor cloacae, Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Streptococcus mutans and Staphylococcus staphylolyticus can be used.

As GTF inhibitors, those derived from Arthrinum sp., Fusarinum sp., Macrophomina sp., Micromonospora sp., Gnomoniella sp., Nodulisporium sp., and Aspergillus sp., can be used, and more specifically, those described in Japanese Patent Application Laid-Open Nos.56-103193, 57-28097, 57-98215 and 57-146587 can be used.

As proteases, those derived from Aspergillus sp., and Bacillus sp., can be used.

As dextranases, those derived from Chaetomium sp., Streptomyces sp., Bacillus sp. and Corynebacterium can be used.

In this invention, each of these synergist components may be used alone or in a combination of one or two.

The caries-preventive composition according to this invention can be prepared and used in various forms applicable to the mouth such as dentifrices (including toothpaste, toothpowder and liquid dentifrice), mouthwashes, dental pastes, gingival massage creams, gargle tablets, troches, chewing gums, ice-creams, whipped creams and the like.

The antibody and the synergist component may be mixed in a given form. Alternatively, the antibody and the synergist component may be jointly used after they are prepared separately.

It is preferred that the quantity of said antibody administered is 0.0001 to 50 g/kg/day. As to the quantity of said synergist component administered, a quantity corresponding to 0.0001 to 1 g/kg/day fluorine for fluorine compounds, a quantity corresponding to 0.0001 to 1 g/kg/day chlorhexidine for chlorhexidine and its salts, a quantity

of 0.0001 to 10 g/kg/day each for lytic enzymes, bacteriocins and glucosyltransferase inhibitors and a quantity of 0.0001 to 5 g/kg/day each for proteases and dextranases are preferably used. The blended amount of the antibody to the oral composition may be in the range of 0.0002 to 10%, preferably 0.002 to 5% by weight of the total weight of the composition. As to the blended amount of the synergist component in the composition, it is preferred that an amount corresponding to 0.0001 to 0.1 wt%, preferably 0.0001 to 0.001 wt% fluorine for fluorine compounds; an amount corresponding to 0.1 to 1000 ppm, preferably 10 to 100 ppm chlorhexidine for chlorhexidine and its salts; and an amount of 0.0001 to 10 wt%, preferably 0.001 to 5 wt% each for lytic enzymes, bacteriocins, glucosyltransferase inhibitors, proteases and dextranases may be blended to the composition.

The oral composition according to this invention may further include additional well-known ingredients depending on the type and form of a particular oral composition. Any desired known ingredients may be mixed with said antibody and synergist component.

In preparing dentifrice compositions, an abrasive may be blended generally in an amount of 5 to 95%, especially 15 to 60% by weight of the composition, including calcium secondary phosphate dihydrate, calcium secondary phosphate anhydrate, calcium primary phosphate, calcium tertiary phosphate, calcium carbonate, calcium

pyrophosphate, insoluble sodium metaphosphate, magnesium tertiary phosphate, magnesium carbonate, calcium sulfate, titanium dioxide, resins, and the like.

In preparing paste-like compositions, typically toothpastes, a binder may be blended generally in an amount of 0.3 to 5% by weight, including sodium carboxymethyl cellulose, methyl cellulose, sodium carboxymethyl hydroxyethyl cellulose, hydroxyethyl cellulose, gum arabic, tragacanth gum, karaya gum, polyvinylalcohol, sodium polyacrylate, carboxyvinyl polymer, polyvinyl pyrrolidone, and the like.

In preparing paste-like and liquid oral compositions, typically toothpastes and mouthwashes, a humectant may be blended generally in an amount of 10 to 70% by weight, including polyethylene glycol, ethylene glycol, sorbitol, glycerol, propylene glycol, 1,3-butylene glycol, xylitol, maltitol, lactitol, and the like.

In addition to the above ingredients, a surface active agent including water soluble salts of alkyl sulfate having 8 to 18 carbon atoms such as sodium laurate and sodium myristate, sodium salts of higher fatty acids, water-soluble salts of sulfonated monoglycerides of higher fatty acids having 10 to 18 carbon atoms in the fatty acid group such as sodium lauryl monoglyceride sulfonate and sodium coconut monoglyceride sulfonate, sodium monoglyceride monosulfates of high r fatty acids, olefin sulfonates, paraffin sulfonates, sodium N-methyl-N-palmitoyl touride,

sodium N-lauroyl sarcosinate, sodium N-lauroyl-β-alanine, stearyl monoglyceride, sucrose fatty acid esters having 12 to 18 carbon atoms in the fatty acid group such as sucrose monolaurate and dilaurate, lactose fatty acid esters, lactitol fatty acid esters, maltitol fatty acid esters, stearic acid monoglyceride, polyoxyethylene sorbitan monolaurate, polyoxyethylene-hardened castor oil, condensates of sorbitan monostearate with approximately 60 moles of ethylene glycol, condensates of ethylene oxide with propylene oxide, and their derivatives such as polyoxyethylene polyoxypropylene monolauryl ester, betaine and amino acid type amphoteric surfactants, and the like may be blended in an amount of 0 to 10%, preferably 0.1 to 5%, more preferably 1 to 2.5% by weight of the composition. A flavor such as an essential oil including peppermint oil and spearmint oil and a flavoring material including *l*-menthol, carvone, eugenol and anethole, a sweetener such as sodium saccharinate, stevioside, neohesperidyldihydrochalcone, glycyrrhizin, perillartine, p-methoxycinnamic aldehyde, a preservative, and the like may be blended in an effective amount.

In this invention, effective ingredients such as mutanase, sorbic acid, alexidine, hinokitiol, cetylpyridinium chloride, alkyl glycine, alkyldiaminoethyl glycinate, allantoin, ε-aminocaproic acid, tranexamic acid, azulene, vitamin E, a water soluble primary or secondary phosphate, a quaternary ammonium compound, sodium chloride

and crude drugs may also be blended in an effective amount.

Other types of compositions may also be prepared by selecting any desired ingredients as usual and mixing them by a conventional procedure.

Examples of the other ingredients for various types or forms of the composition are shown in the following Examples.

Paste-like and liquid oral compositions may generally have a pH ranging from 5 to 10, but not limited thereto.

The caries-preventive composition according to this invention, owing to the combination of said antibody and said synergist component, can efficiently suppress the formation of plaque caused by Streptococcus mutans, thereby excellently preventing the formation of dental caries.

Examples of this invention will be given in the following although this invention is not restricted to them.

EXAMPLE 1

Antisera and mother's milks were obtained by using the following antigens according to the following method.

(1) Antigens

Streptococcus mutans NCTC10449

Bacteria grown in the external solution obtained by the dialysis of BHI medium, after being washed, were treated with formalin before being supplied for use.

Cell-wall fraction of Streptococcus mutans NCTC10449

The fraction prepared according to the method of Bleiweis et al. (J. Bacteriol., 88, 1198-1200, 1964) was supplied for use.

Fibrous substance fraction of Streptococcus mutans NCTC10449

The fraction prepared according to the method of J. Van Hoate et al. (Arch. Oral. Bio., 16, 1131-1141, 1971) and Tsurumizu et al (Jap. J. Bacteriology, 38, (1) 471, 1983) were supplied for use.

Glucosyltransferase fraction of Streptococcus mutans NCTC10449

The fraction prepared according to the method of Inoue et al. (Microbial Aspects of dental caries Vol. III, 665-682, 1976 [Information Retrieval Inc.]) was supplied for use.

Protein antigen fraction of Streptococcus mutans NCTC10449

The fraction prepared according to the method of Lehner et al. (J. General Microbiology, 122, 217-225, 1981) was supplied for use.

(2) Preparation of Antiserum and Mother's Milk

Said antigen was mixed with Freund's complete adjuvant, and a pregnant goat, horse, cow or rabbit was immunized with the thus prepared mixture. After the animal was immunized three times with the mixture of said antigen and Freund's incomplete adjuvant before its delivery, the

colostrum was collected after the delivery. As to the antiserum, after the animal was immunized four times in the same manner as above, the blood was collected and coagulated, and supernatant solution obtained by centrifuging the coagulated blood was used as a sample.

An antibody is prepared by adding ammonium sulfate to the antiserum to saturate it at the level of 40%, separating the obtained precipitate by centrifugation, and dialyzing the precipitate against physiological saline, and the inner solution was used as a sample.

Next, the colonizing tests of Streptococcus mutans in the mouth were conducted according to the following method by using said antiserum and mother's milk as well as a fluorine compound, a chlorhexidine salt, a lytic enzyme, a bacteriocin, GTF inhibitors, a protease and a dextranase used as synergist components.

(3) Colonization of Streptococcus mutans in the Mouth

After male hamsters of five week old were divided into groups each consisting of five individuals, each hamster was inoculated with 1×10^8 bacteria of Streptococcus mutans of the NCTC10449 strain. From the day of the inoculation, drinking water containing the effective components (said antiserum or milk and the synergist component) was administered to each hamster. One week and four weeks after the start of the administration, the teeth of each hamster were rubbed with a cotton ball before it is immersed in a small amount of physiological saline to

disperse bacteria homogeneously in it. After a given amount of the thus obtained solution was scattered on the BHI plate medium and the mitis salivalius plate medium, the number of whole bacteria and the number of the colonies of Streptococcus mutans were counted. The number of Streptococcus mutans was indicated by the number of Streptococcus mutans per 10,000 whole bacteria. The concentration of antiserum or mother's milk in the drinking water was adjusted to 0.025%. As to the concentration of the synergist component, it was adjusted to 0.05% for a fluorine compound (NaF), 0.005% for a chlorhexidine salt (chlorhexidine gluconate), 0.05% for a lytic enzyme, 0.01% each for a bacteriocin, a GTF inhibitor and a protease and 0.005% for a dextranase.

For comparison, the same experiments were conducted without jointly using antiserum or mother's milk and the synergist component by adding antiserum or mother's milk alone, by adding the synergist component alone and by adding none of antiserum, mother's milk and the synergist component (Control).

The results obtained by using the fluorine compound (NaF) as the synergist component are indicated in Table 1; those obtained by using chlorhexidine gluconate (CHX), in Table 2; those obtained by using the lytic enzyme, in Table 3; those obtained by using the bacteriocin, in Table 4; those obtained by using the GTF inhibitors, in Table 5; those obtained by using the protease, in Table

6; and those obtained by using the dextranase, in Table 7.

Table 1

Samples Added	Number of S. mutans bacteria 1 week after	Number of S. mutans bacteria 4 weeks after
Control	3890	4250
Goat anti-whole-bacteria serum	2178	1467
" + NaF	1945	297
Goat anti-GTF serum	1828	1510
" + NaF	1556	212
Goat anti-whole-bacteria mother's milk	1984	1382
" + NaF	1945	170
Goat anti-cell-wall serum	1750	1340
" + NaF	1750	255
Goat anti-protein serum	1984	1255
" + NaF	1945	85
Goat anti-fibrous-substance milk	1945	1340
" + NaF	1711	127
NaF alone	3112	2040

Table 2

Samples Added	Number of S. mutans bacteria 1 week after	Number of S. mutans bacteria 4 weeks after
Control	3890	4250
Antibody from equine anti-whole-bacteria serum	2139	1425
" + CHX	2139	212
Antibody from equine anti-GTF serum	1789	1297
" + CHX	1634	340
Equine anti-whole-bacteria mother's milk	1945	1340
" + CHX	1984	170
Equine anti-cell-wall serum	2022	1425
" + CHX	1867	297
Equine anti-protein serum	2023	1425
" + CHX	1945	212
Equine anti-fibrous-substance milk	1867	1383
" + CHX	1134	85
CHX alone	3112	2975

Table 3

Samples Added	Number of S. mutans bacteria 1 week after	Number of S. mutans bacteria 4 weeks after
Control	3890	4250
Bovine anti-whole-bacteria serum	2178	1425
" + Lytic enzyme	1945	255
Bovine anti-GTF serum	1828	1382
" + Lytic enzyme	1556	340
Bovine anti-whole-bacteria mother's milk	2023	1298
" + Lytic enzyme	1945	127
Antibody from bovine anti-cell-wall serum	2139	1255
" + Lytic enzyme	1867	85
Bovine anti-protein serum	1556	1085
" + Lytic enzyme	1556	42
Bovine anti-fibrous substance milk	2334	1298
" + Lytic enzyme	1984	85
Lytic enzyme alone	3112	3485

Note) As the lytic enzyme, one obtained from Streptomyces globisporus was used.

Table 4

Samples Added	Number of S. mutans bacteria 1 week after	Number of S. mutans bacteria 4 weeks after
Control	3890	4250
Rabbit anti-whole-bacteria serum	2178	1383
" + Bacteriocin	2100	298
Rabbit anti-GTF serum	1556	1467
" + Bacteriocin	1634	255
Rabbit anti-whole-bacteria mother's milk	1945	1510
" + Bacteriocin	1945	213
Rabbit anti-cell-wall serum	2023	1595
" + Bacteriocin	1634	170
Rabbit anti-protein serum	1945	1298
" + Bacteriocin	1751	128
Rabbit anti-fibrous-substance milk	2178	1383
" + Bacteriocin	1751	128
Bacteriocin alone	2723	3060

Note) As the bacteriocin, one obtained from Streptococcus L-1, microbial technology research laboratory trust number 3220, was used.

Table 5

Samples Added	Number of S. mutans bacteria 1 week after	Number of S. mutans bacteria 4 weeks after
Control	3890	4250
Goat anti-whole-bacteria serum	2188	1425
" + GTF inhibitor A	2100	212
Goat anti-GTF serum	1867	1297
" + GTF inhibitor A	1789	176
Goat anti-whole-bacteria mother's milk	1945	1340
" + GTF inhibitor B	1828	85
Goat anti-cell-wall serum	2022	1425
" + GTF inhibitor A	1945	340
Goat anti-protein serum	1945	1297
" + GTF inhibitor C	1906	255
Goat anti-fibrous substance milk	2178	1383
" + GTF inhibitor A	1751	128
GTF inhibitor A alone	2723	3485
GTF inhibitor B alone	3112	3400
GTF inhibitor C alone	2995	3315

Note) GTF inhibitor A was obtained from Aspergillus terreus;
GTF inhibitor B, from Arthrinum sp. M 5071; and
GTF inhibitor C, from Micromonospora sp. SF-2259.

Table 6

Samples Added	Number of S. mutans bacteria 1 week after	Number of S. mutans bacteria 4 weeks after
Control	3890	4250
Equine anti-whole-bacteria serum	2334	1297
" + Protease	2022	85
Equine anti-GTF serum	1867	1340
" + Protease	1789	85
Equine anti-whole-bacteria mother's milk	2022	1382
" + Protease	1867	42
Equine anti-cell-wall serum	2178	1510
" + Protease	2022	170
Equine anti-protein serum	1945	1552
" + Protease	1711	42
Equine anti-fibrous-substance milk	2100	1340
" + Protease	1634	170
Protease alone	3034	3655

Note) The protease used is derived from Aspergillus sp.

Table 7

Samples Added	Number of S. mutans bacteria 1 week after	Number of S. mutans bacteria 4 weeks after
Control	3890	4250
Bovine anti-whole-bacteria serum	2334	1297
" + Dextra-nase	2100	212
Bovine anti-GTF serum	2022	1510
" + Dextra-nase	1789	212
Bovine anti-whole-bacteria mother's milk	2139	1552
" + Dextra-nase	1945	340
Bovine anti-cell-wall serum	2139	1595
" + Dextra-nase	1828	85
Bovine anti-protein serum	2022	1297
" + Dextra-nase	1983	170
Bovine anti-fibrous substance milk	1867	1383
" + Dextra-nase	1634	85
Dextranase alone	2022	1275

Note) The dextranase used is derived from Chetomium sp.

From the results indicated in Tables 1 to 7, it is found that the combination of the antiserum or mother's milk and the synergist component according to this invention excellently suppresses the colonization of Streptococcus mutans.

EXAMPLE 2 Toothpaste

Calcium secondary phosphate dihydrate	50.0%
Glycerol	20.0
Sodium carboxymethylcellulose	1.0
Sodium lauryl sulfate	1.5
Sodium lauroyl sarcosinate	0.5
Flavor	1.0
Sodium saccharinate	0.1
Water	Balance
	100.0%

The above components were blended with 0.1% or 0.2% antibody of goat whole-bacteria and 0.1% sodium fluoride, 0.01% chlorhexidine gluconate, 0.1% a lytic enzyme, 0.01% a bacteriocin, 0.001% a protease, 0.1% GTF inhibitor-A or 0.25% (3000 units/g) a dextranase.

EXAMPLE 3 Toothpaste

Calcium secondary phosphate	50.0%
Sorbitol	10.0
Glycerol	10.0
Sodium carboxymethylcellulose	1.0
Sodium lauryl sulfate	2.0
Flavor	1.0

Sodium saccharinate	0.1
Ethanol	2.0
Mutanase	0.1
Water	Balance
	100.0%

The above components were blended with 0.1% bovine anti-cell-wall serum and 0.3% sodium monofluorophosphate, 0.01% chlorhexidine gluconate, 0.05% a lytic enzyme, 0.02% a bacteriocin, 0.001% a protease, 0.1% GTF inhibitor-C or 0.25% a dextranase.

EXAMPLE 4 Toothpaste

Calcium carbonate	50.0%
Glycerol	20.0
Sodium carboxymethylcellulose	1.5
~~Sodium carboxymethylcellulose~~	~~1.0~~
Sodium lauryl sulfate	0.5
Sucrose monolaurate	2.0
Flavor	1.0
Sodium saccharinate	0.1
Water	Balance
	100.0%

The above components were blended with 0.05% bovine anti-GTF mother's milk and 0.1% sodium fluoride, 0.005% chlorhexidine gluconate, 0.1% a lytic enzyme, 0.01% a bacteriocin, 0.001% a protease, 0.1% GTF inhibitor-B or 0.25% a dextranase.

EXAMPLE 5 Toothpaste

Calcium secondary phosphate dihydrate	50.0%
Glycerol	20.0
Sodium carboxymethylcellulose	2.0
Sodium lauryl sulfate	2.0
Flavor	1.0
Sodium saccharinate	0.1
Water	Balance
	100.0%

The above components were blended with 0.1% equine anti-protein serum and 0.1% stannous fluoride, 0.01% chlorhexidine gluconate, 0.05% a lytic enzyme, 0.01% a bacteriocin, 0.001% a protease, 0.1% a GTF inhibitor or 0.25% a dextranase.

EXAMPLE 6 Toothpaste

Calcium secondary phosphate dihydrate	30.0%
Glycerol	30.0
Sorbitol	20.0
Sodium carboxymethylcellulose	1.0
Sodium lauryl sulfate	2.0
Flavor	1.0
Sodium saccharinate	0.1
Ethanol	2.0
Water	Balance
	100.0%

The above components were blended with 0.1% sheep anti-protein serum and 0.1% stannous fluoride, 0.01%

chlorhexidine gluconate, 0.1% a lytic enzyme, 0.01% a bacteriocin, 0.0001% a protease, 0.1% GTF inhibitor-A or 0.17% (2000 units/g) a dextranase.

EXAMPLE 7 Toothpowder

Calcium secondary phosphate dihydrate	50.0%
Calcium carbonate	30.0
Glycerol	10.0
α-olefin sulfonate	1.0
Flavor	1.0
Sodium saccharinate	0.1
Dextran	0.5
Water	Balance
	100.0%

The above components were blended with 0.1% sheep anti-fibrous-substance serum and 0.1% sodium monofluorophosphate and 0.1% sodium fluoride, 0.01% chlorhexidine gluconate, 0.05% a lytic enzyme, 0.001% a bacteriocin, 0.0001% a protease, 0.1% GTF inhibitor or 0.17% a dextranase.

EXAMPLE 8 Liquid Dentifrice

Sodium polyacrylate	50.0%
Glycerol	30.0
Flavor	0.9
Sodium saccharinate	0.1
Ethanol	3.0
Linolic acid	0.05

Water	Balance
	100.0%

The above components were blended with 0.01% or 0.02% goat anti-GTF mother's milk and 0.01% or 0.02% goat anti-protein mother's milk and 0.02% sodium fluoride, 0.05% chlorhexidine gluconate, 0.05% a lytic enzyme, 0.001% a bacteriocin, 0.002% a protease, 0.02% GTF inhibitor-A or 0.25% a dextranase.

EXAMPLE 9 Mouthwash

Ethanol	20.0%
Flavor	1.0
Sodium saccharinate	0.05
Sucrose monolaurate	0.3
Water	Balance
	100.0%

The above components were blended with 0.1% goat anti-GTF serum and 0.1% sodium monofluorophosphate and 0.01% stannous fluoride, 0.01% chlorhexidine gluconate, 0.05% a lytic enzyme, 0.001% a bacteriocin, 0.01% a protease, 0.01% GTF inhibitor-B or 0.25% a dextranase.

EXAMPLE 10 Mouthwash (tablet)

Sodium hydrogencarbonate	54.0%
Sodium secondary phosphate	10.0
Polyethylene glycol	3.0
Citric acid	17.0
Sodium sulfate (anhydrous)	13.6
Flavor	2.0

Oleic acid	0.1
	100.0%

The above components were blended with 0.1% rabbit anti-GTF serum and 0.1% sodium monofluorophosphate and 0.05% sodium fluoride, 0.05% chlorhexidine gluconate, 0.05% a lytic enzyme, 0.01% a bacteriocin, 0.005% a protease, 0.05% GTF inhibitor-A or 0.25% a dextranase.

The tablet is used by dissolving 0.5 g of the tablet into 50 ml of water.

EXAMPLE 11 Gingival Massage Cream

White petrolatum	8.0
Propylene glycol	4.0
Stearyl alcohol	8.0
Polyethylene glycol 4000	25.0
Polyethylene glycol 400	37.0
Sucrose stearate	0.5
Water	Balance
	100.0%

The above components were blended with 0.5% bovine anti-fibrous-substance mother's milk and 0.5% sodium fluoride, 0.01% chlorhexidine gluconate, 0.05% a lytic enzyme, 0.01% a bacteriocin, 0.0% a protease, 0.5% GTF inhibitor-A or 0.25% a dextranase.

EXAMPLE 12 Chewing Gum

Gum base	43.85%
Calcium carbonate	2.0
Starch syrup	15.0

Sugar	30.0
Sucrose palmitate	1.0
Fructose	4.0
Maltose	3.0
Flavor	1.0
	100.0%

The above components were blended with 0.1% bovine anti-whole-bacterial ~~body~~ mother's milk and 0.1% stannous fluoride, 0.01% chlorhexidine gluconate, 0.1% a lytic enzyme, 0.01% a bacteriocin, 0.001% a protease, 0.1% GTF inhibitor-C or 0.25% a dextranase.

EXAMPLE 13 Troche

Gum arabic	6.0
Grape sugar	75.0
Flavor	0.2
l-menthol	0.1
Spearmint oil	0.1
Sodium ascorbate	0.1
Water	Balance
	100.0%

The above components were blended with 0.05% or 0.1% goat anti-protein serum and 0.05% sodium fluoride, 0.01% chlorhexidine gluconate, 0.05% a lytic enzyme, 0.01% a bacteriocin, 0.005% a protease, 0.1% GTF inhibitor-C or 0.25% a dextranase.

EXAMPLE 14 Dental Paste

Polyoxyethylene monostearate	2.0%
Sorbitan monooleate	2.0
Cetyl alcohol	2.0
Palmityl alcohol	3.0
Propylene glycol	15.0
Carboxymethylcellulose	5.0
Saccharine	0.2
Peppermint oil	0.5
Spearmint oil	0.5
Lysozyme chloride	5000 units/g
Water	Balance
	100.0%

The above components were blended with 0.05% or 0.1% equine anti-GTF serum and 0.05% sodium fluoride, 0.01% chlorhexidine hydrochloride, 0.05% a lytic enzyme, 0.01% a bacteriocin, 0.005% a protease, 0.1% GTF inhibitor-A or 0.25% a dextranase.

EXAMPLE 15 Dental Paste

Glyceryl monolaurate	3.0%
Oleyl alcohol	5.0
Polyethylene glycol	15.0
White petrolatum	3.0
N-palmitoyl monosodium glutamate	0.5
Hydroxyethylcellulose	5.0
Tocopheryl acetate	0.1
Sodium saccharinate	0.2

Japanese peppermint oil	0.7
Carvone	0.5
Anethole	0.3
Eugenol	0.1
Water	Balance
	100.0%

The above components were blended with 0.025% or 0.05% rabbit anti-fibrous-substance serum and 0.05% sodium fluoride, 0.01% chlorhexidine hydrochloride, 0.05% a lytic enzyme, 0.001% a bacteriocin, 0.0025% a protease, 0.05% GTF inhibitor-B or 0.25% a dextranase.

EXAMPLE 16 Ice-cream

Cream (fat content, 50%)	16.84%
Milk (fat content, 3.7%) *	42.65
Defatted evaporated milk	24.24
Sugar	11.25
Corn syrup	4.65
Stabilizer	0.35
	100.0%

* : Containing 0.5% bovine anti-fibrous-substance mother's milk

The above components were blended with 0.05% a lytic enzyme or 0.05% a bacteriocin.

EXAMPLE 17 Ice-cream

Cream (fat content, 59%)	16.84%
Milk (fat content, 3.7%) *	42.65
Defatted evaporated milk	24.24

Sugar	11.25
Corn syrup	4.65
Stabilizer	0.35
	100.00%

* : Containing 3% bovine anti-fibrous-substance mother's milk

The above components were blended with 0.001% a protease, 0.002% GTF inhibitor-C or 0.021% (250 units/g) a dextranase.

EXAMPLE 18 Ice-cream

Cream (fat content, 40%)	31.54%
Milk (fat content, 3.7%) **	37.16
Defatted evaporated milk	15.08
Sugar	11.25
Corn syrup	4.67
Stabilizer	0.30
	100.00%

** : containing 5% bovine anti-protein mother's milk.

The above components were blended with 0.05% a lytic enzyme, 0.05% a bacteriocin, 0.001% a protease, 0.1% GTF inhibitor-A or 0.42% (5000 units/g) a dextranase.

CLAIMS :

1. A caries-preventive composition comprising

an antibody obtained by immunizing a mammal with at least one antigen selected from the group consisting of Streptococcus mutans, its cell-wall fraction, fibrous substance fraction, glucosyltransferase fraction and protein antigen fraction, and

a synergist selected from the group consisting of fluorine compounds, chlorhexidine and its salts, lytic enzymes, bacteriocins, glucosyltransferase inhibitors, proteases and dextranases.

2. The composition as claimed in claim 1, wherein Streptococcus mutans is one belonging to the serotype C separated from human mouth.

3. The composition as claimed in claim 1 or 2, wherein the antibody is obtained from equine antiserum.

4. The composition as claimed in claim 1 or 2, wherein the antibody is obtained from bovine antiserum or milk.

5. The composition as claimed in any one of claims 1 to 4, wherein the antibody is prepared from the precipitate obtained by saturating the antiserum or milk with ammonium sulfate at the level of not more than 40%.

6. The composition as claimed in any preceding claim, wherein the blending amount of the antibody is in the range of 0.0002 to 10% by weight of the composition.

7. The composition as claimed in any preceding claim, wherein the synergist comprises a fluorine compound selected from the group consisting of monofluorophosphates, alkali-metal fluorides and fluorides containing stannous tin.

8. The composition as claimed in claim 7, wherein the fluorine compound is selected from the group consisting of sodium monofluorophosphate, sodium fluoride, and stannous fluoride.

9. The composition as claimed in any one of claims 1, 7 and 8, wherein the synergist comprises a fluorine compound and the blending amount of the fluoride compound is in the range of 0.0001 to 0.1% by weight of the composition as fluorine.

10. The composition as claimed in any one of claims 1 to 6, wherein the synergist comprises a chlorhexidine salt selected from the group consisting of chlorhexidine hydrochloride and chlorhexidine gluconate.

11. The composition as claimed in claim 1 or claim 10, wherein the synergist comprises chlohexidine or a chlorhexi-dine salt and the blending amount of chlorhexidine or its salt is in the range of 0.1 to 1000 ppm.

12. The composition as claimed in any one of claims 1 to 6, including a synergist selected from the group consisting of lytic enzymes, bacteriocins, glucosyltransferase inhibitors, proteases and dextranases and the blending amount thereof is in the range of 0.0001 to 10% by weight of the composition.

13. The composition as claimed in any preceding claim wherein the composition is prepared as a dentifrice, a mouthwash, an oral paste or a gingival massage cream.

14. The composition as claimed in any one of claims 10 to 12, wherein the composition is prepared as a troche or a chewing gum.

15. The composition as claimed in claim 12, wherein the composition is prepared as an ice cream.

European Patent Office

EUROPEAN SEARCH REPORT

0140498
Application number

EP 84305462.8

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
D,Y	GB - A - 1 505 513 (STOLLE RESEARCH AND DEVELOPMENT CO) * Page 1, line 57 - page 2, line 58 * --	1,2,4, 13-15	A 61 K 7/16 A 61 K 39/40
Y	WO - A1 - 82/04 396 (THE SECRETARY OF STATE FOR SOCIAL SERVICES IN HER BRITANNIC MAJESTY'S GOVERNMENT) * Claims 5-9; abstract * --	1,2,13	
Y	GB - A - 2 033 223 (THE SECRETARY OF STATE FOR SOCIAL SERVICES) * Claims 17-21; abstract * --	1,2,13	
Y	DE - A1 - 2 757 290 (BLENDAX) * Pages 6,7 * --	1,7-10 12,13	
Y	GB - A - 2 008 948 (ANIC S.P.A.) * Claims 1-4 * --	1,12, 13	
Y	CHEMICAL ABSTRACTS, vol. 96, no. 11, March 15, 1982, Columbus, Ohio, USA TOYO JOZO CO. LTD "Glucosyltransferase inhibitor M 5071" page 467, right column, abstract-no. 84 106g & Jpn. Kokai Tokkyo Koho JP 81,103, 193 --	1,12	

TECHNICAL FIELDS SEARCHED (Int. Cl.4)

A 61 K 7/00
A 61 K 39/00

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
VIENNA	16-11-1984	IRMLER

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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Y	CHEMICAL ABSTRACTS, vol. 98, no. 3, January 17, 1983, Columbus, Ohio, USA ENDO, AKIRA "Physiologically active mutastein" page 414, right column, abstract-no. 15 482f & Eur. Pat. Appl. EP 59,918 ----	1,12	

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X : particularly relevant if taken alone
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